Biological Synopsis of the Invasive Tunicate *Styela clava* (Herdman 1881)

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ABSTRACT

Clarke, C.L., and Therriault, T.W. 2007. Biological synopsis of the invasive tunicate *Styela clava* (Herdman 1881). Can. Manuscr. Rep. Fish. Aquat. Sci. 2807: vi + 23 p.

Styela clava (Herdman 1881), commonly known as the clubbed tunicate, is a fast-growing, subtidal fouling species. It is a prolific breeder and highly efficient suspension feeder. Its native range is the Sea of Okhotsk south to Shanghai. It is an aggressive invader that has successfully established populations in temperate waters worldwide. Characterized by a discontinuous, disjunct distribution like many invaders, *S. clava* was first discovered outside its native range in North America on the Californian coast in 1932. The most probable introduction vectors include ship fouling and oyster transfer. This species is now present on both the Atlantic and Pacific coasts of Canada, though it has not been detected in Arctic waters. *Styela clava* is restricted to southern British Columbia on the Pacific coast and waters surrounding Prince Edward Island on the Atlantic coast. It is considered a major pest for shellfish aquaculture, smothering target species and fouling gear and equipment. This species is tolerant of a wide range of temperature and salinity, though it seems to prefer protected waters. Its distribution is thought to be limited by temperature, salinity, wave exposure and depth. All Canadian waters up to 25m depth, with salinities ranging between 20 and 32‰ and temperatures ranging between 2 and 23°C are at risk of invasion by *S. clava*. This species has limited larval dispersal, due to the short planktonic phase and therefore, vector traffic may be a more important predictor of distribution than habitat characteristics. Further study is required to determine the source populations and the key dispersal vectors available to this invader on both the Pacific and Atlantic coasts of Canada.

RÉSUMÉ

Clarke, C.L., and Therriault, T.W. 2007. Biological synopsis of the invasive tunicate *Styela clava* (Herdman 1881). Can. Manuscr. Rep. Fish. Aquat. Sci. 2807: vi + 23 p.

Styela clava (Herdman, 1881), couramment appelée ascidie plissée, est une salissure marine à croissance rapide qui vit en milieu infralittoral. Elle est un reproducteur prolifique et un suspensivore très efficace. L'espèce est indigène de la mer d'Okhotsk, au sud de Shanghai. Elle est un envahisseur agressif qui s'est établi avec succès dans des eaux tempérées partout dans le monde. Son aire de répartition est discontinue et distincte, comme celle de nombreux envahisseurs. Elle a été observée pour la première fois à l'extérieur de son aire de répartition d'origine en 1932, sur la côte de la Californie. Les vecteurs d'introduction les plus probables comprennent les bateaux infestés et les transferts d'huîtres. L'ascidie plissée est maintenant présente sur les côtes de l'Atlantique et du Pacifique du Canada, mais elle n'a toujours pas été observée dans les eaux arctiques. Sur la côte Ouest, *Styela clava* n'est présente que dans le Sud de la Colombie-Britannique, tandis que sur la côte Est, elle n'est présente que dans les eaux entourant l'Île-du-Prince-Édouard. Elle est considérée comme une espèce nuisible importante dans le domaine de la conchyliculture puisqu'elle étouffe les espèces cibles et salit les engins et l'équipement. Elle tolère une vaste gamme de températures et de salinités, mais elle semble préférer les eaux protégées. La température, la salinité, l'exposition aux vagues et la profondeur seraient les facteurs qui déterminent l'aire de répartition de l'espèce. Toutes les eaux canadiennes de moins de 25 m de profondeur, où la salinité varie entre 20 et 32 ‰ et où la température varie entre 2 et 23 °C, sont menacées par *S. clava*. La dispersion des larves de l'ascidie plissée est limitée en raison de la courte phase planctonique de l'espèce et, par conséquent, les déplacements des vecteurs pourraient constituer un indice plus important que les caractéristiques de l'habitat pour prévoir la répartition de l'espèce. Il est nécessaire de mener des études plus poussées afin d'identifier les populations sources et les vecteurs de dispersion importants sur les côtes Est et Ouest du Canada.

1.0 INTRODUCTION

The introduction of nonindigenous species is considered the second most important risk to native biodiversity, behind habitat loss (Elton 1958; Carlton 1989; Sala 2000). The Government of Canada (2004) defines invasive alien species as "those harmful alien species whose introduction or spread threatens the environment, the economy or society, including human health". Globally, invasive species are considered responsible for the extinction of over 110 vertebrate species and affect almost every ecosystem they invade. In addition to biodiversity loss and the alteration of ecosystem function, many nonindigenous species have considerable economic costs. The characterised cost of only ten nonindigenous species to Canada was estimated at $187 million per year (Colautti *et al.* 2006). The rate of new introductions has increased exponentially over the last 100 years and shows little sign of easing (Levings *et al.* 2002).

There are at least six species of invasive tunicates known in the marine waters of Canada. They include the solitary *Styela clava* Herdman, *Ciona intestinalis* Linnaeus, *Molgula manhattensis* DeKay and the colonial *Didemnum* sp., *Botryllus schlosseri* Pallas, and *Botrylloides violaceus* Oka. At present, *C. intestinalis* only is found on the Atlantic and Arctic coasts of Canada while *Didemnum* sp. only has been confirmed on the Pacific coast. All three colonial tunicate species and the solitary tunicates, *Styela clava* and *Ciona intestinalis,* are the focus of risk assessments for Canada currently underway. The focus of the current background document is *Styela clava.* This species is a major invasion threat for all temperate marine ecosystems and has successfully invaded North America, Europe, Australia and New Zealand. *Styela clava* is an aggressive invader; affecting native biodiversity by replacing the native competitive dominants in the community. This species is a major pest of concern for the shellfish industry and causes an asthmatic condition in Japanese workers removing the species from shellfish products. The projected economic impact of this species on shellfish production for Canada alone was estimated at between $34,000 and $88,000 per year (Colautti *et al.* 2006).

Here we present the distribution of this species at regional, national and international scales and review the state of knowledge of the biology and ecology of *S. clava* and discuss the impacts, and possible vectors and pathways. Also, we discuss the broad environmental tolerances of this species, especially temperature and salinity, which suggest most Canadian waters could be suitable for this species.

1.1 Name and Classification

Kingdom: Animalia
Phylum: Chordata
Subphylum: Tunicata
Class: Ascidicea
Order: Stolidobranchia
Family: Styelidae

Genus: *Styela*
Species: *clava*

Scientific name: *Styela clava* Herdman 1881
Common names: clubbed tunicate, leathery sea squirt, rough sea squirt
Earlier synonyms: *Bostryorchis clava* Redikorzev 1916; *Styela barnharti* Ritter and Forsyth 1917; *Styela mammiculata* Carlisle 1954; *Styela clava clava* Nishikawa 1991

1.2 Description

Styela clava is a large, solitary tunicate with a cylindrical body resembling a club (Figures 1, 2). The body proper is covered by a tough, brown, leathery tunic that tapers to a short stalk or peduncle. The stalk terminates in a disc-like holdfast. The siphons are short, positioned close together and anteriorly directed. The siphonal apertures have four longitudinal brown or purple stripes alternating with paler bands. Siphons and apertures can be clearly seen in an actively feeding animal but may be inconspicuous in exposed, stressed or preserved specimens. The tunic is folded in irregular longitudinal folds as well as transversely wrinkled and grooved, especially at the anterior end. Large rounded bumps called tubercles are present on the adult tunic. Larger specimens often are covered with epibionts such as corals, algae, hydroids, sponges and smaller conspecifics (Lutzen 1999). Smaller specimens are yellowish-brown and are attached directly to the substrate with no holdfast or peduncle. *Styela clava* possesses two to five slender gonads on the left side and between four and nine on the right side. Small lobed testes are attached along the length of the ovaries (Lambert 2003). Sexually mature adults range in size from 90 to 160mm (Kott 1985; Lutzen 1999). *Styela clava* can be confused with *S. montereyensis* but the latter can be distinguished by its regular longitudinal grooves and one recurved siphon (as *S. barnharti* in Van Name 1945; Lambert 2003).

2.0 DISTRIBUTION

2.1 Native distribution

Styela clava is native to the northwest Pacific Ocean. It was originally described by Herdman (1881) from dredged specimens from the Sea of Okhotsk (Abbott and Johnson 1972). Its native range (Figure 3) extends from the Sea of Okhotsk, through southern Siberia, Japan, Korea and northern China, south to Shanghai (Cohen 2005).

2.2 Non-native distribution (excluding Canada)

Styela clava now occurs worldwide in temperate marine waters including both the western and eastern United States (US), United Kingdom (UK), Europe, Asia, and

Australia (Figure 4). The timing of reported introductions worldwide is presented in Table 1. It is unknown which of the three previously identified introduction pathways were responsible for each region's invasion but introduction pathways will be discussed in more detail in Section 5.0. Regardless, these non-native populations can now be considered secondary sources for the introduction of *S. clava* to Canada. The earliest recorded introduction was Newport, California in 1933 but probably took place in the late 1920s (Abbott and Johnson 1972). Hull fouling or Japanese oyster transport to Elkhorn Sound are the most probable vectors (Lambert and Lambert 2003; Cohen 2005). This species now ranges from Baja, Mexico north to Washington State, US and into southern British Columbia (see Section 2.3.1 for more detail) (Lambert and Lambert 2003). *Styela clava* has been detected at three sites in Puget Sound, Washington: Blaine, Neah Bay and Pleasant Harbor and 11 confirmed sites in BC (Figure 5). It is unclear from the pattern of introduction which pathway(s) are responsible for the spread of this species in Pacific North America.

Styela clava was next discovered in Plymouth, Devon, UK in 1953 (Carlisle 1954; Houghton and Millar 1960). It is believed to have been introduced on the hulls of warships returning from the Korean War after 1951 (Eno *et al.* 1997). A small population was discovered in Dieppe, France in 1968 (Monniot 1970 IN Lutzen 1999). *Styela clava* spread to Limfjord Harbour, Denmark in the mid-1960s (Lutzen and Sorensen 1993). In 1971, this species was discovered in Cork Harbour, Ireland (Guiry and Guiry 1973). Subsequently, *S. clava* was found in the Netherlands in 1974 (Cohen 2005), Belgium in 1986 (Lutzen 1999), Germany in 1997 (Lutzen 1999), and Spain in 2004 (Davis and Davis 2004). It seems likely that most of the more recent sightings are a result of secondary spread and/or stepping stone introductions, although reintroduction from its native range cannot be ruled out.

In the southern hemisphere, populations currently are restricted to large ports with high levels of commercial shipping activities. An isolated population was found in Hobson's Bay, Australia in 1972 and has not spread further (Holmes 1976). *Styela clava* was recently discovered by visiting scientists in Auckland and Christchurch, New Zealand (Eno *et al.* 1997). Extensive surveys have confirmed the presence of this species on both the south and north island, and in many harbours and marinas surrounding Auckland (Eno *et al.* 1997; Lutzen 1999). The reason for the isolation of the Australian population and the seemingly rapid spread of the New Zealand population is unknown.

On the Atlantic coast of North America, *S. clava* was first found in low abundance in 1970 in a marina in Beverly, Massachusetts (Berman *et al.* 1992). The path of introduction was hypothesized to be from Japan via Europe sometime during the 1960s (Berman *et al.* 1992). This pathway was inferred because the primary trade route is from Europe rather than Asia and many of the introduced species in this region are of European origin (Pederson *et al.* 2005). However, it has not been ruled out that this species came directly from Asia either within or associated with live oyster shipments. *Styela clava* was first found in New York in 1972 (Cohen 2005). In New Hampshire, this species was first reported in 1985 in Portsmouth Harbor (Berman *et al.* 1992). It had spread to Rhode Island by 1980, and Connecticut and Maine by 1988 (Cohen 2005). The

first report of *S. clava* in Atlantic Canada was from the Montague/Brudenell/St. Mary's Bay area in Prince Edward Island (PEI) in 1998 (N. MacNair, DFO, PEI, unpublished data).

2.3 Distribution in Canada

2.3.1 Current Distribution

Styela clava has been present in Pacific Canadian waters since at least 1994 when specimens were collected at Nanaimo, Vancouver Island (Lambert and Lambert 1998). There is a stable but isolated population at French Creek Marina, Vancouver Island (Lambert and Lambert 1998). Two additional records of *S. clava* in BC from the literature are from Nanoose Bay and Maple Bay, both on Vancouver Island (Lambert and Lambert 2003). Informal surveys of aquaculture leases for invasive tunicates were recently conducted by Fisheries & Oceans Canada (D. Paltzat, DFO, Nanaimo, unpublished data). The results of these surveys showed that *S. clava* was more widespread in BC than initially thought (Figure 5). The species was found at five aquaculture sites, all in southern BC. The invaded areas included Baynes Sound, Okeover Inlet, Cortes Island, West Redonda, and Quadra Island. It is important to note that *S. clava* was not detected at all sites surveyed in the Strait of Georgia and the west coast of Vancouver Island. Settlement plates set out by Fisheries & Oceans Canada also recorded the presence of this species at two additional sites: Deep Bay Marina and the wharf at the Institute for Ocean Sciences in Sidney. Sites further north in the central and north coast regions of BC have not yet been surveyed so other populations could exist. There has been no effort to search for *S. clava* in Arctic Canada but it was not detected during general exploratory surveys in Alaska (Lambert 2003). It is probable that the temperature regime in this region limits *S. clava*'s establishment success even if suitable vectors were available. It has been hypothesized that *S. clava* was introduced to Pacific North America with Japanese oyster shipments or hull fouling in the mid 20th century and then dispersed through intra-regional vectors such as aquaculture activities or hull fouling on commercial or recreational watercraft (Cohen 2005). However, it remains unclear if the Canadian populations resulted from the primary introduction from Asia or via other secondary pathways. The Strait of Georgia is one of the most invaded marine or estuarine waterbody in BC (Levings *et al.* 2002) and therefore *S. clava* may have been introduced by a number of possible vectors.

In Atlantic Canada, *S. clava* has remained restricted to waters surrounding Prince Edward Island (PEI). The Atlantic coast of North America mode of introduction is believed to be commercial shipping to Georgetown Harbour (Berman *et al.* 1992) with subsequent spread through a number of potential vectors, including aquaculture activities, fisheries gear fouling, commercial shipping and recreational boating. The distribution of this species on the east coast is presented in Figure 6. Scientists in eastern Canada have been actively conducting surveys for invasive tunicates but there have been no reports of *S. clava* from New Brunswick, Nova Scotia, Newfoundland or Quebec thus far. This species is fairly widespread to the south and has been present in the northeast United

States since 1970 and is now widely distributed from New York to Maine (Berman *et al.* 1992; Lutzen 1999).

2.3.2 Potential Distribution

The potential distribution of an invasive species is the complex interplay of two factors: the presence of suitable habitat and available vectors to access those habitats. There are a number of ecological factors that have been used to describe the habitat preferences of ascidian species worldwide. Lambert and Lambert (2003) proposed that ascidian distribution may be limited by salinity, temperature, water movement, anthropogenic bacteria and harbour size. The salinity and temperature tolerances of this species will be discussed in Section 3.2: Physiological Tolerances. Similar to other filter feeders, ascidian habitat choice is a balance between sufficient water movement to supply food and nutrients and avoiding high degrees of disturbance from wave action and currents (Lambert and Lambert 2003). *Styela clava* prefers calm, protected waters or sheltered microhabitats on more exposed coasts (Lambert and Lambert 2003). Harbour size has been suggested as a factor limiting ascidian distribution because invasive ascidian species diversity is higher in larger harbours (Lambert and Lambert 2003). Large harbours may have more stable physical characteristics, with fewer deviations in salinity and temperature than smaller ones. However, for invasive ascidians, the effect of harbour size also may be related to vector strength (propagule pressure), in that larger harbours would have more ships from a greater variety of source countries.

Based on the physiological tolerances discussed in Section 3.2 and the above information the predicted habitat for this species in Canada would meet these conditions: 1) maximum depth of 25m, 2) temperature between 2°C and 23°C, and 3) salinity between 20‰ and 32‰. However, since this animal readily colonizes artificial substrates, natural depth might not be a useful predictor. A variety of habitats supporting introduced *S. clava* populations (e.g., harbours, aquaculture sites, pilings, ropes, and docks, etc.) each create shallow water habitat in places where it would not occur naturally.

3.0 BIOLOGY AND NATURAL HISTORY

3.1 Age and Growth

Immature *S. clava* grow rapidly, at an average rate of 10-15mm per month in a California population (Morris *et al.* 1980). Small specimens have no distinct peduncle and the body is attached directly to the substrate (Lutzen 1999; Cohen 2005). Growth slows as the animal reaches sexual maturity (85-90mm), typically within five to six months in California (Parker *et al.* 1999) and 10 months in Denmark (Lutzen 1999). The observed regional differences in growth and age at sexual maturity may be related to temperature differences or food supply. Growth is reduced over winter, when water temperatures are

lower. In Europe, maximum length, including the peduncle, is 125mm, although it has been recorded to 160mm in extreme cases (Lutzen 1999). There have been no estimates of mortality for this species but the life span is a maximum of three years (Morris *et al.* 1980; Lambert and Lambert 1998).

3.2 Physiological Tolerances

It has been suggested that *S. clava* is such a successful invader because of its ability to tolerate of a wide range of environmental factors, a trait common among many invasive species. For example, *S. clava* was able to survive temperatures as low as 2°C in the Netherlands (Lambert and Lambert 2003) and as high as 23°C in the United Kingdom (Holmes 1969). However, it is important to note that this species was unable to reproduce at temperatures less than 15°C (Eno *et al.* 1997). This species has behavioural adaptations to handle adverse conditions or environmental stressors (e.g. hyposalinity). *Styela clava* can close its siphons for extended periods of time, with adult specimens able to withstand temporary drops in salinity, to as low as 8‰ (Sims 1984; Lutzen 1999). However, Davis and Davis (2004) reported that this species was not found in areas where the salinity was consistently lower than 20‰ suggesting a lower threshold of salinity tolerance does exist. Sims (1984) described this species as possessing limited hyperosmotic capability, supporting its absence in highly estuarine conditions which would be greater than 20‰ (Lutzen 1999). Larvae metamorphose in salinities between 20‰ and 32‰, while 18‰ is deleterious to the larvae (Kashenko 1996 IN Lutzen 1999). Therefore the salinity tolerance of *S. clava* is consistent with the distribution pattern described by Davis and Davis (2004).

3.3 Reproduction

Styela clava is hermaphroditic but not self-fertile as the male and female gonads mature at different times (Wallace 1961; Kott 1985). This species is oviparous and fertilisation is external. Upon maturity, this species has the ability to spawn every 24 hours (Biosecurity New Zealand 2005). It is unknown whether this species undergoes synchronous spawning but spawning is believed to be a response to light and water temperature (Parker *et al.* 1999; Lambert 2005a) with no spawning at temperatures less than 15°C (Eno *et al.* 1997). An upper temperature limit for spawning has not been determined. There are significant regional differences in the reproductive season exhibited by *S. clava*, which may be caused by temperature differences. For example, in California spawning takes place between early spring and autumn (Cohen 2005) while in New England larvae were found between June and November with peaks between July and October (Osman and Whitlatch 1999). In Ireland, spawning peaked between August and October, but occurred mainly in September and October (Parker *et al.* 1999). The population structure in Cork Harbour, England, also showed evidence for spawning between August and October.

The fecundity of *S. clava* has not been determined but we assume it would be consistent with other solitary ascidians. Egg production for introduced *Ciona intestinalis* was

estimated at 1,000-10,000 eggs per day per individual (Carver *et al.* 2003). *Styela clava* eggs and larvae are lecithotrophic and remain planktonic for 24-28 hours at 20°C before settling, attaching and metamorphosing on the substrate (Cohen 2005). Studies indicate that the maximum settlement distance of ascidians in general is 10m from the adult and most dispersal is much less (Osman and Whitlatch 1995; McHenry 2005). The degeneration of the larval ocellus was hypothesized to reduce the larval capacity for site selectivity (Kott 1985). Upon release, tunicate larvae are initially negatively geotactic and positively phototactic but this behaviour is reversed as the larvae becomes competent to settle (McHenry 2005). Most settlement occurs during slack water (NIMPIS 2002), which may be an adaptation for successful metamorphosis or a side effect of the interaction between particles and water movement. Chemical cues, such as those created by the presence of conspecifics also may influence the timing and location of larval settlement (McHenry 2005).

3.4 Feeding and Diet

Styela clava is an extremely efficient suspension feeder, preying on phytoplankton, zooplankton and other suspended organic materials (NIMPIS 2002). Large solitary ascidians are capable of a high rate of filtration (Lambert and Lambert 2003). Mature *S. clava* kept in moving water at 10°C filtered 78-175ml of water/g/dry weight of body (Morris *et al.* 1980). To feed, *S. clava* pumps water in through the oral aperture, strains the food particles through the branchial filter and pumps the filtered water out through the atrial aperture (Cohen 2005). The pore size on the ascidian mucus sheet covering the branchial filter is approximately 0.2 µm (Lambert and Lambert 1998), small enough to remove bacteria and organic molecules. Osman *et al.* (1989) found that *S. clava* predation removed 96% of oyster larvae in the water column. Ascidians can play a positive role in some areas by filtering and sequestering heavy metals and other pollutants from the water (Lambert and Lambert 1998).

3.5 Habitat

Styela clava is a low intertidal to subtidal fouling species. It has been recorded at 40m depth but more typically occurs between 15-25m (Lutzen 1999). This species can withstand tidal immersion in some microhabitats (e.g., under rocks) and is most common in sheltered habitats with low wave action, such as inlets, bays, harbours and marinas (Lutzen 1999). It also has been found on high energy outer coasts on the partially protected surfaces of rocks or pilings. *Styela clava* can be found attached to a range of artificial substrates, including pier pilings, jetty walls, concrete structures, submerged ropes, buoys, floating docks, and vessel hulls (Lutzen 1999; NIMPIS 2002). *Styela clava* is a secondary settler, settling on substrates already fouled by other species. It is found on natural substrates as well, including rocks and bivalve beds and epiphytically on *Crassostrea gigas, Mytilus edulis,* and *Sargassum muticum* (Lutzen 1999; NIMPIS 2002).

This species occurs in lower densities on natural substrates, 50-100/m^2 and an order of magnitude higher on artificial surfaces, 500-1000/m^2 (see Lutzen 1999 for review). Floating artificial substrates form a unique habitat that may be highly suitable for invading *S. clava* populations, causing the significant difference in density compared to natural habitats (Lambert 2005a). The characteristics that promote invasion on these surfaces may include a lack of benthic predators and low structural complexity. In addition, the introduction of a new substrate free from the established communities of native species offers valuable new space to invaders. Once a foothold has been established, the short dispersal distance may allow *S. clava* to generate high density local populations.

3.6 Interspecific Interactions

3.6.1 Predation

Styela clava experiences ontogenetic and size-selective shifts in predation pressure. Larvae are susceptible to predation by a range of planktivorous predators. Newly settled ascidian juveniles become prey for mobile carnivorous or omnivorous invertebrates and fish (reviewed in Lambert 2005b). For example, in New England, juveniles are susceptible to high mortality (almost 100%) from snails such as *Mitrella lunata* and fish such as *Tautogolabrus adspersus* (Osman and Whitlatch 1999; NIMPIS 2002). The presence of these predators seemed to prevent the establishment of *S. clava* populations in these areas (Osman and Whitlatch 1995). Adult *Styela clava* have a tough, leathery tunic and no predators have been recorded for north-western Europe (Lutzen 1999; NIMPIS 2002; Cohen 2005). Predators recorded for other adult solitary ascidians include flatworms, gastropods, sea stars, crabs, fish and birds (Lambert 2005b). Many ascidians deter predation and fouling by producing noxious secondary metabolites. However, *S. clava* may employ a different strategy: the thickening, tubercles and folds of the tunic promote fouling by other organisms such that individuals become cryptic (Lambert 2005b). The rapid growth rate allows them to escape predation through size at an earlier age than comparable native species (Osman and Whitlatch 1995).

3.6.2 Competition

Styela clava is a highly aggressive competitor for space and food. It grows to a considerable length, so that even medium-sized specimens stand above other co-occurring sessile species. This may confer an advantage in filtering food from the water column and preying upon the larvae of its competitors (Lutzen 1999). Experiments by Osman *et al.* (1989) showed that *S. clava* is capable of greatly reducing the local settlement rate of oysters by preying on their planktonic larvae. The presence of *S. clava* reduced the settlement of most other species, except for barnacles and spirobiid polychaetes (Osman and Whitlatch 1999). The introduction and increase of *S. clava* in southern England was matched by a concurrent decline in the population of the local ascidian, *Ciona intestinalis* (Lutzen 1999). *Styela clava* has effectively replaced the indigenous *Pyura haustor* and *Ascidia ceratodes* as the dominant ascidian species in

southern California (Lambert and Lambert 1998). *Styela clava* is able to colonize the bare surfaces left behind with the death of earlier settled individuals of the native species, *Ascidiella aspersa* (Holmes 1969). In addition, *S. clava* recruitment does not appear to be deterred by native species assemblages in New England (Whitlatch *et al.* 1995).

In some regions, native species offer some control of *S. clava* introductions and these may act to slow or reduce the impact of this invader. *Styela clava* may be successfully out-competed by large monospecific populations or settlement events of native species, swamping potential recruitment surfaces. On pier pilings in the Yarra River (Australia), where barnacles dominate, *S. clava* was absent, but present in marine waters nearby (Cohen *et al.* 2001 IN NIMPIS 2002). However, this remains circumstantial evidence as this observation may be a response to competition with barnacles or a result of differences in salinity. In Limfjord harbours and marinas, large mussel spat settlement events may prevent *S. clava* from returning to an area where it was previously abundant (Lutzen 1999). In Europe, *S. clava* may be at a competitive disadvantage because it spawns later than the local species, with which it competes for space (Osman *et al.* 1989; Lutzen 1999).

4.0 USE BY HUMANS

Styela clava is a highly prized delicacy in southern Korea, consumed under the local popular name "mideuduck" (Morris *et al.* 1980). It is actively farmed in Korea and exported to Europe and North America for consumption (Lambert 2005b).

5.0 VECTORS AND PATHWAYS

There are three major pathways by which invasive species can be introduced to new areas: 1) direct introduction, 2) secondary spread, and 3) stepping stone introduction. Direct introduction is the transfer of the species from its native range to the new location. Secondary spread is the larval or physical dispersal to adjacent areas within the introduced range, also known as range expansion. In this case we would expect to see a slow rate of spread from the epicentre in relation to life history characteristics of the invading species. The mechanism for secondary spread is usually larval dispersal but also can be the result of physical dislodgement of individuals or asexual reproduction in colonial species. Stepping stone introduction is the transfer of the species from the original introduction site to new areas through a series of intermediate areas (stepping stones) and is governed by the vectors involved.

In the case of *S. clava* all three pathways are possibilities for invasion. The first recorded introduction to California was likely a direct introduction from Japan. Subsequent records of the species in neighbouring harbours may be the result of secondary spread. The sightings at further distances may have resulted from stepping stone introductions or additional direct introductions from its native range. On the Atlantic coast of North

America, stepping stone introduction is believed to be responsible for the original sightings of *S. clava*, moving from the native range to Europe and finally to Massachusetts. The pathway of introduction to PEI in unknown but its spread within the region is likely the result of a combination of secondary spread and stepping stone introductions.

This species exhibits a patchy distribution within its introduced range. In Britain, areas colonised by *S. clava* were interspersed with apparently suitable sites, suggesting that natural larval dispersal does not necessarily lead to colonisation of neighbouring sites (Davis and Davis 2004). A similar patchy distribution has been described for *S. clava* in the eastern US where this species occurred at high densities at some sites but was rare or completely absent at similar sites nearby (Osman and Whitlatch 1995). Microhabitat differences such as local circulation patterns and the presence/absence of local predators and competitors in seemingly equally suitable habitats can cause patchy distributions. Predation on small recruits and juveniles can completely eliminate this species from some areas, even though transplanted adults may survive there (Osman and Whitlatch 1995).

Styela clava has a short larval period and therefore, a limited ability to disperse planktonically. This characteristic may contribute greatly to its patchy distribution in newly invaded habitats. In light of the limited larval dispersal, *S. clava*'s distribution is likely related directly to the availability of suitable vectors that provide physical transport of adults to new areas. Osman and Whitlatch (1995) showed that transplanted animals survived and grew at similar rates to the original site, supporting the supposition that dispersal may be vector-limited. The discontinuous distribution of this species worldwide also supports this hypothesis. This species appears at irregular intervals both spatially and temporally and does not seem to spread within a region without the aid of intra-regional vectors.

A number of vectors have been proposed for the introduction of *S. clava* worldwide. The most probable include ship fouling, live oyster transfer and fisheries gear fouling. Ballast water introduction has largely been ruled out for this species because of the short larval period, however ship transit times are being reduced and *S. clava* larvae may survive shorter intraregional journeys successfully. There is no direct evidence for these vectors but circumstantial evidence exists based on the timing and geographical distribution of reported sightings. For example, its introduction to Plymouth Harbour in 1953 coincided with the arrival of warships returning from the Korean War and therefore hull fouling was proposed as the vector. The introduction in Elkhorn Sound, California in the late 1920s was consistent with the timing of the Japanese oyster import but also could have been attributed to shipping. The rapid spread of *S. clava* within PEI may be attributable to the movement of aquaculture and fisheries equipment between sites.

Genetic analyses are required to resolve the questions regarding both the pathways and vectors governing the spread of *S. clava*. If we can determine the most important vectors and pathways responsible for past introductions we can then predict where we should concentrate our efforts in prevention, eradication and mitigation.

6.0 IMPACTS ASSOCIATED WITH INTRODUCTION

6.1 Impacts on Native Diversity

The competitive superiority of *S. clava* over mussels and oysters presents a major problem for fisheries and shellfish aquaculture. This species competes for food and space with other filter feeders and benthic species but because it does not produce a shell it can grow faster and reach maturity earlier than competing bivalves. *Styela clava* has the ability to reduce settlement rates of co-occurring species locally but seems to have little effect on post-settlement community dynamics (Whitlatch *et al.* 1995). In Japan, Denmark and eastern Canada, *S. clava* has been reported as a major pest of oyster and mussel farms (Cohen 2005). In many places, this species has replaced local ascidians as the dominant member of shallow, protected habitats worldwide (Whitlatch *et al.* 1995; Lutzen 1999; Lambert and Lambert 2003).

6.2 Impacts on Industry

This species' ability to settle on a range of artificial surfaces causes dense fouling of fishing gear, moorings, and ropes and is difficult and time-consuming to remove. In Denmark, *S. clava* is a major gear fouling problem for the cod, flounder and eel fisheries. In Prince Edward Island, mussel lines and floating docks have been weighed down by heavy growth of *S. clava,* making them difficult to remove from the water. The tremendous density and abundance of this species in PEI is considered a serious threat to the long-term economic viability of the shellfish industry. Over 500,000 kg of *Styela clava* is removed in PEI each year (G. Lambert, University of Washington, Seattle, pers. comm.). The projected economic impact has been conservatively estimated to be between $34,000 and $88,000 per year (Colautti *et al.* 2006) and could increase if this species spreads. Hull fouling increases drag for the shipping industry and consequently increases fuel and cleaning costs. In Japan, this species is responsible for an asthmatic condition in workers employed shucking oysters (NIMPIS 2002; Cohen 2005).

6.3 Treatment & Mitigation

Solitary ascidians are considered easier to control than their colonial counterparts because they do not reproduce asexually. However, recent efforts to eradicate this species from Pleasant Harbor Marina, Washington, by a large volunteer group failed. The group physically removed over half a ton but the invasive population was much more extensive than initially thought (Droscher 2006).

Treatment options for *S. clava*-fouled oysters include various combinations of salinity, temperature and air exposure (Eno *et al.* 1997). Dipping oysters in brine for 5 minutes at 14°C followed by 30 minutes of air exposure caused total mortality of *S. clava* with no observable effect on the oysters (Minchin and Duggan 1988). In PEI growers are using

an acetic acid solution (vinegar) to treat the mussel lines with limited success. However, these types of chemical treatments are not always feasible for large-scale, open environment aquaculture operations. Farm operators in Nova Scotia have had limited success with a high pressure water spray mechanism that punctures the tunic of *Ciona intestinalis* thereby killing the organism with approximately 75% effectiveness in removing this species from mussel lines (P. Darnell, Indian Point Mussels, Nova Scotia, pers. comm.). A similar treatment may be effective on other large solitary tunicates such as *S. clava* if the water pressure is great enough to puncture the tough tunic of this species without damaging the shellfish.

The New Zealand Aquaculture Council recently released a code of practice for preventing the spread of *S. clava*. This voluntary code asks farmers to actively look for specimens of this species, remove and report their occurrence and to prevent its movement from affected areas by ensuring both equipment and vessels are clean before moving to clear areas (New Zealand Aquaculture Council 2005).

7.0 KNOWLEDGE GAPS

An analysis of the vector pathways should be undertaken to determine the historical vectors responsible for introductions, both to Canada and worldwide. Commercial shipping and live adult oyster transport are both means of potential introduction that could be compared to Canadian and worldwide spread of *S. clava* in an attempt to better understand the mechanism(s) of introduction. Genetic (DNA) analyses of *S. clava* specimens collected worldwide are currently underway by Dr. John Bishop at Plymouth, UK, in an attempt to determine invasion pathways (Lambert 2005c).

To prevent further introduction and spread of *S. clava* in Canada additional work is required to determine the source(s) of introductions – the native range in Asia or the numerous non-native populations around the world. Genetic testing could be used to determine the source of introductions. When combined with analyses of possible vectors, a potential forecast of "hotspots" might be possible. In Pacific Canada, *S. clava* could be introduced directly from Asia, or from non-native populations in the US or the small localized populations known from southern waters through intraregional transport. In Atlantic Canada, this species may be introduced from established populations in Europe, via intraregional shipping from the US or secondary spread from PEI. An additional concern is the movement of aquaculture equipment and animals within and to unaffected areas outside PEI.

Following the completion of the studies mentioned above, mitigation efforts could then be focused on those vectors and source populations that pose the greatest risk to the introduction of *S. clava* to unaffected Canadian waters.

TABLES

Table 1: Introduction of *Styela clava* worldwide with year of earliest sighting in each region and the reference in which it was first recorded.

Year	Place of earliest sighting	Reference
1933	Pacific USA	Abbott & Johnson, 1976
1953	England	Carlisle, 1954
1968	France	Coughlan 1969
1970	Atlantic USA	Berman *et al.*, 1992
1976	Australia	Holmes, 1976
1993	Pacific Canada	Lambert, 2003
1998	Atlantic Canada	MacNair, DFO, 2006, pers. comm.
2005	New Zealand	Biosecurity New Zealand, 2005a

FIGURES

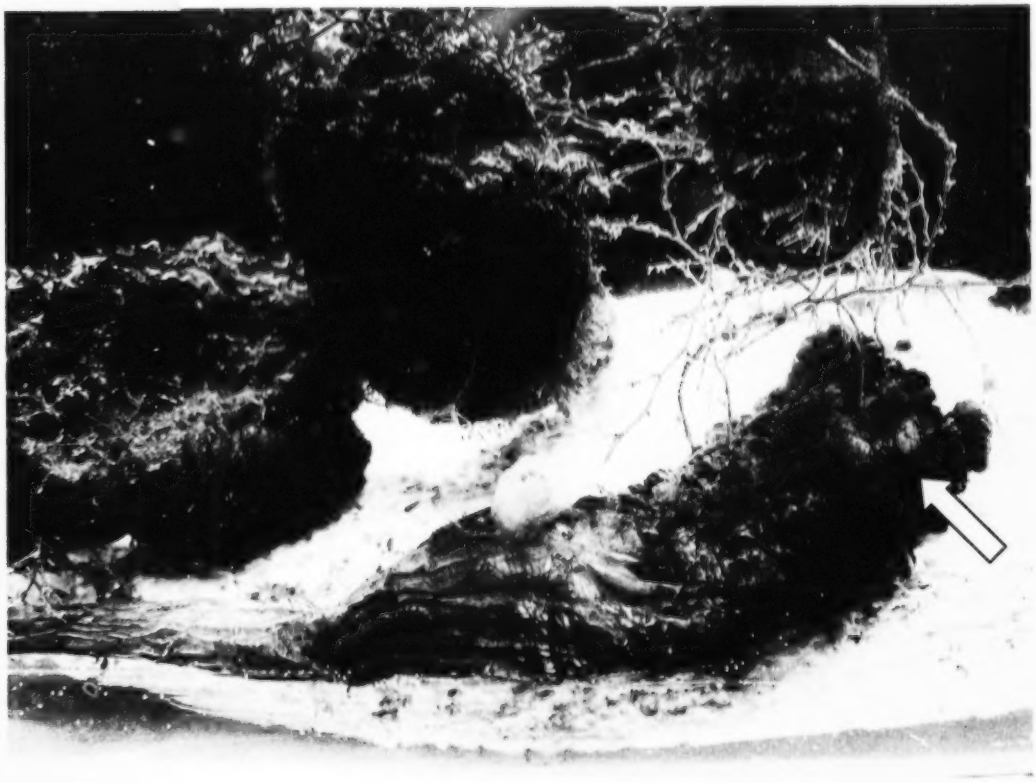

Figure 1: *Styela clava* specimen at lower right of photograph (arrow points toward anterior end). Photo supplied by Jim Boutillier, DFO, Nanaimo.

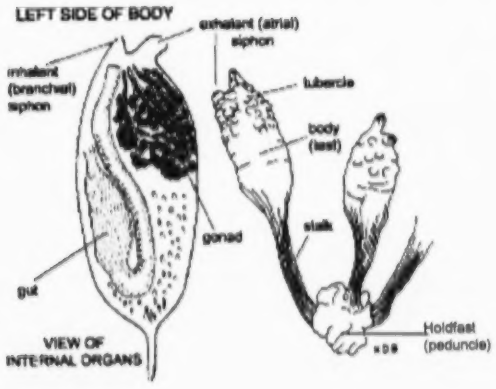

Figure 2: Diagram of *Styela clava* illustrating internal and external structures. Adapted from NIMPIS 2002; Kott 1985; Christiansen and Thomsen 1981.

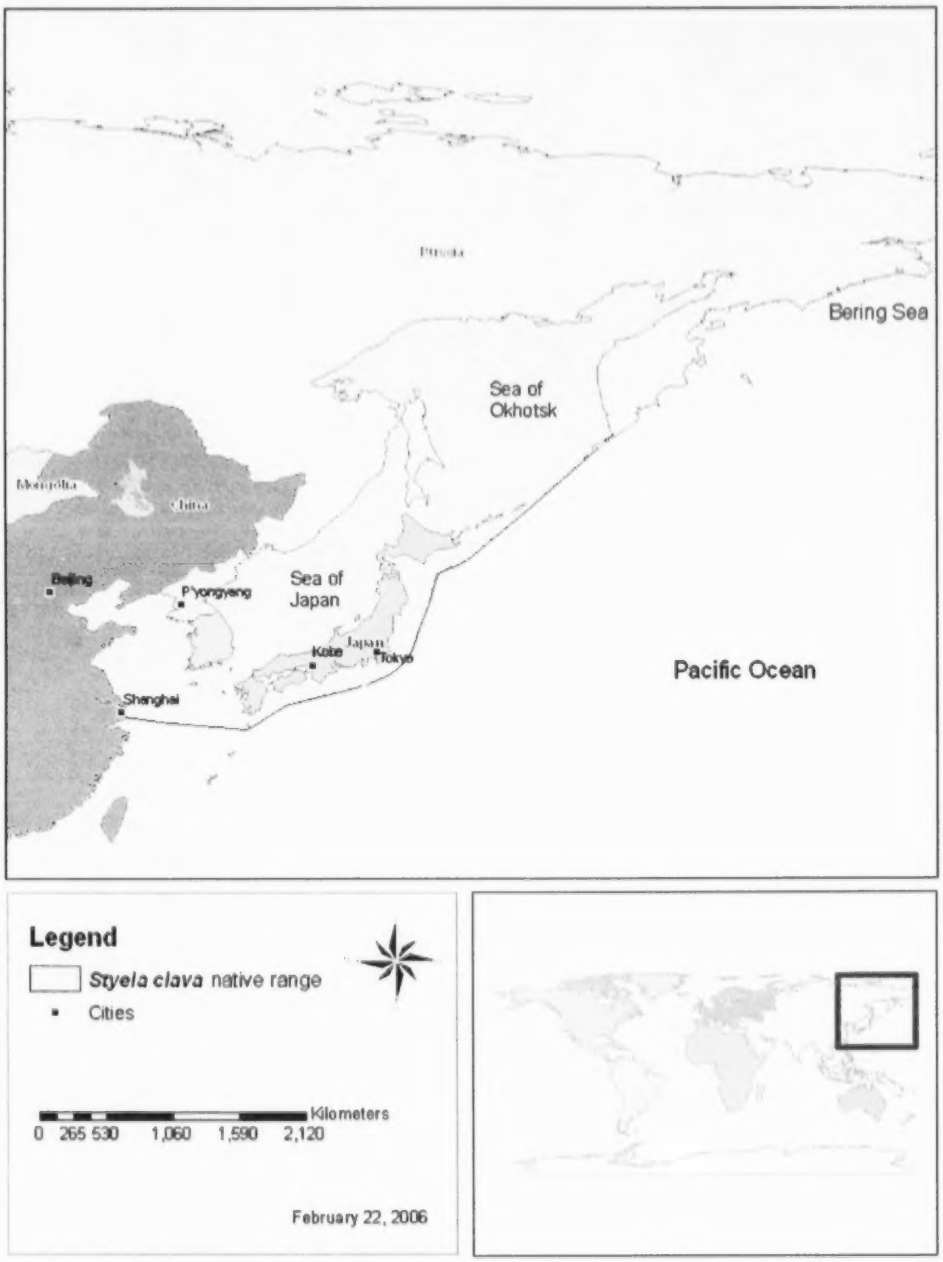

Figure 3: Northwest Pacific, native range of the ascidian *Styela clava*. Inset map shows extent of map on world continents.

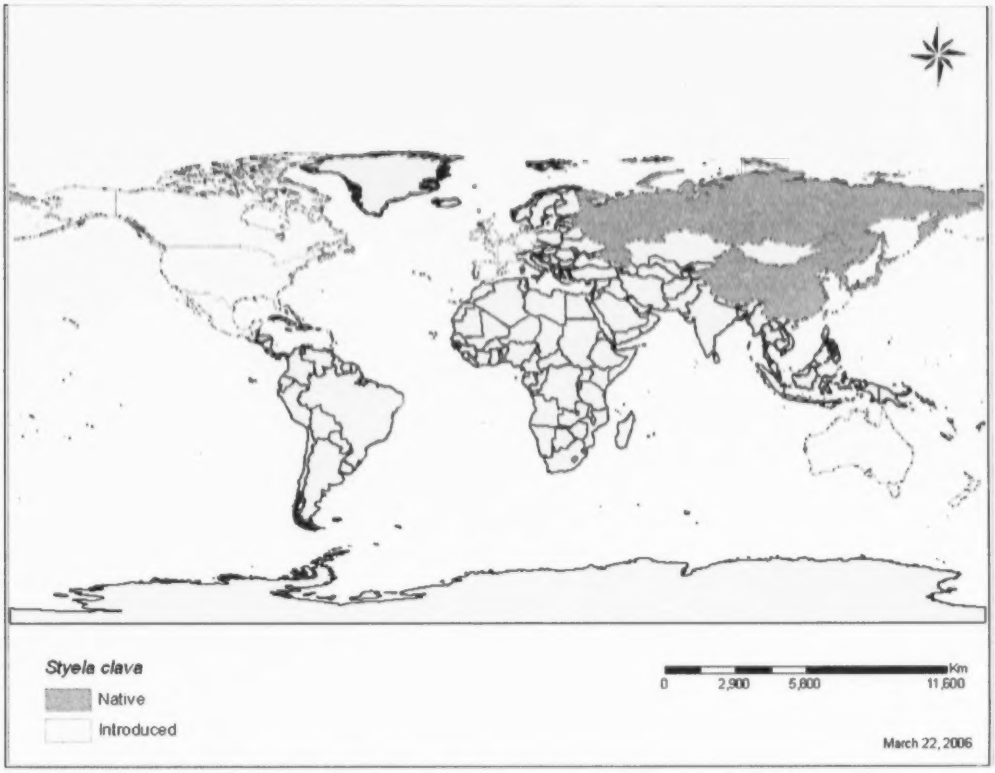

Figure 4: Countries where *Styela clava* occurs worldwide: the native range is in red and introduced in yellow. *Styela clava* is not necessarily found over the entire coast line of those countries highlighted.

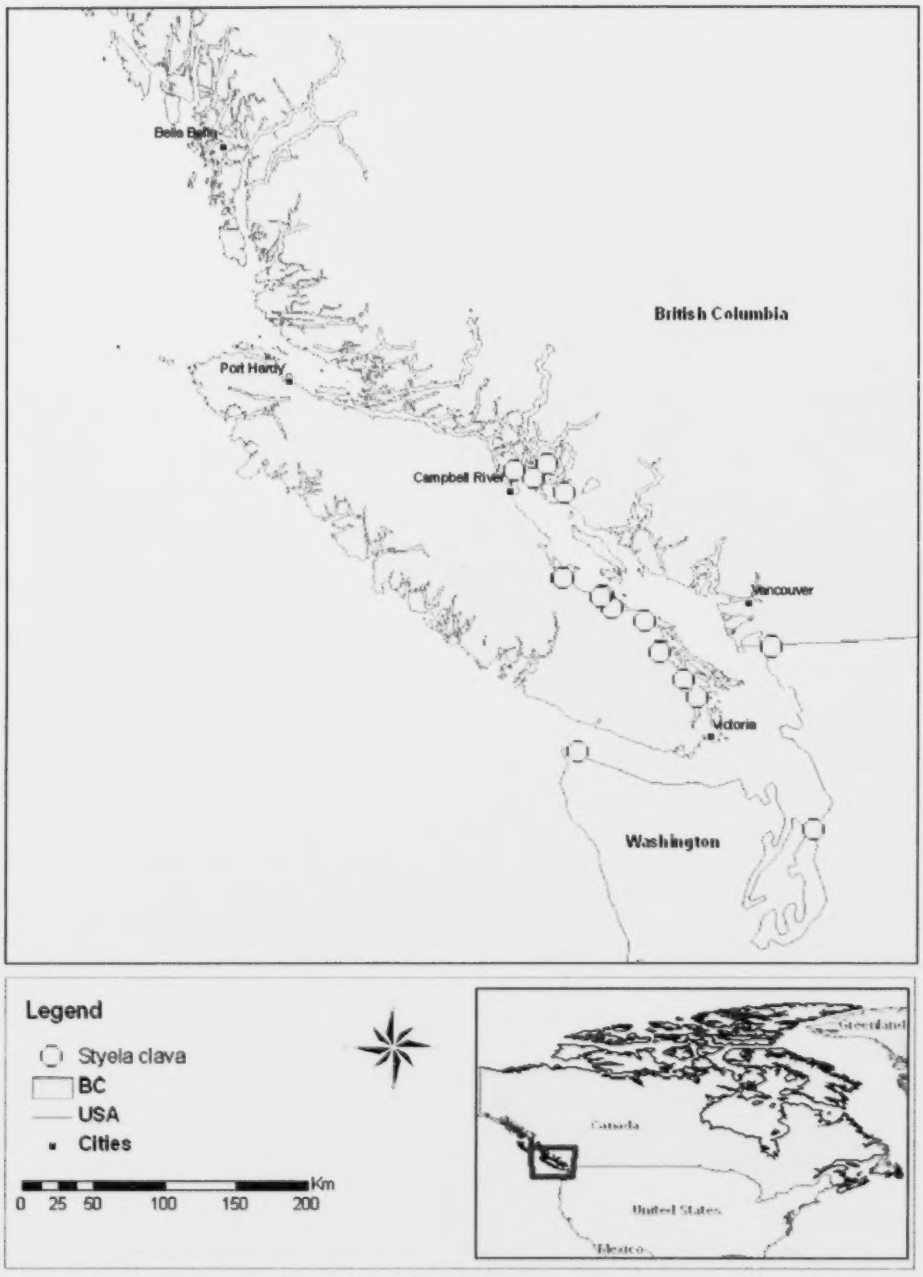

Figure 5: Distribution of *Styela clava* in Pacific Canada based on reference records, aquaculture site surveys and collector plate settlement experiments by DFO.

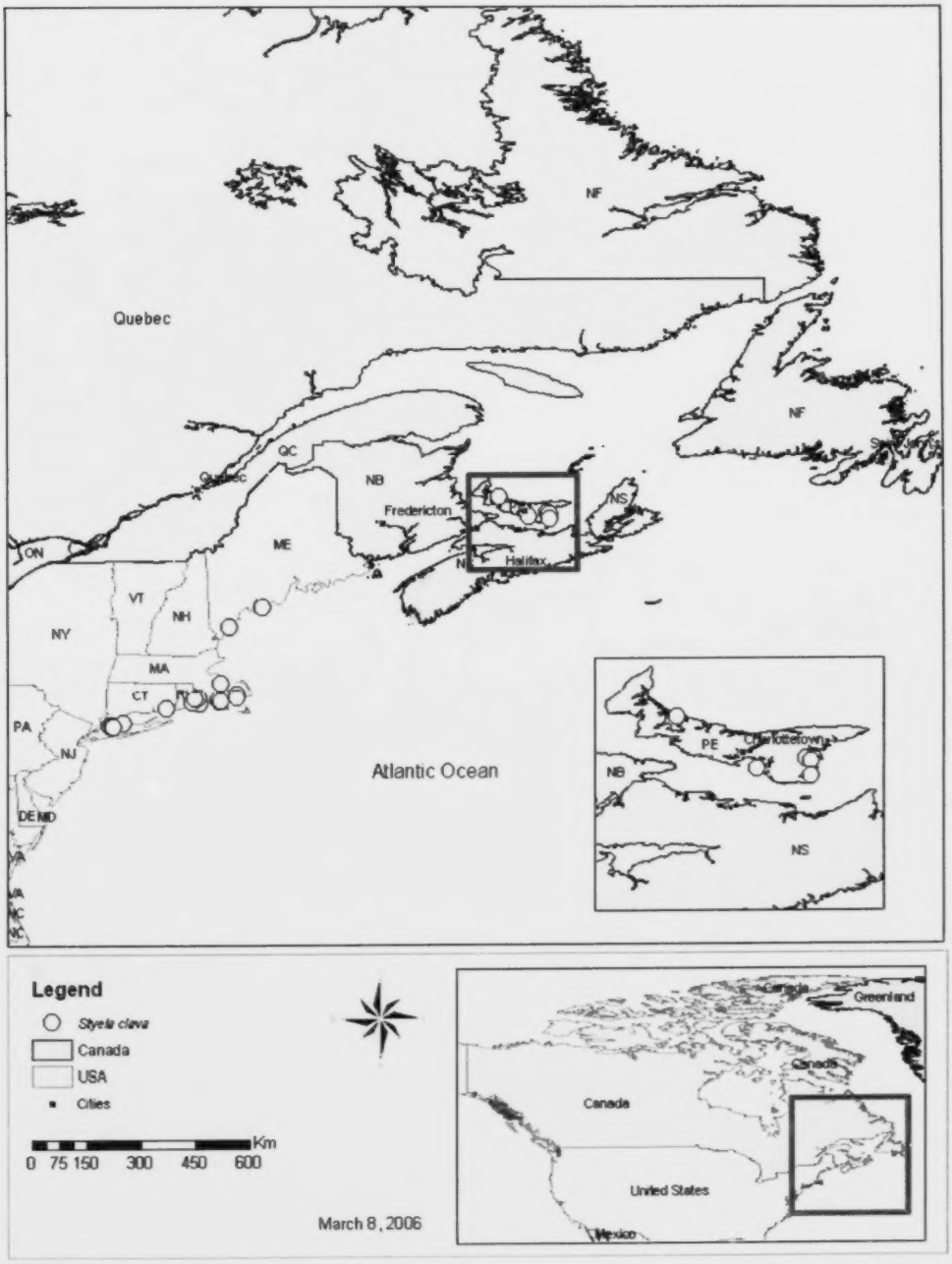

Figure 6: Distribution of *Styela clava* on the east coast of North America. Small inset map shows distribution on Prince Edward Island. Large inset map shows reference box within Canada.

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